Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 3, 2015

Original Article

DESIGN AND EVALUATION OF FORSKOLIN BUCCAL MUCOADHESIVE MICROSPHERES

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Received: 18 Mar 2015, Revised and Accepted: 18 Jun 2015

ABSTRACT

Objective: The objective of the present study was to develop buccal mucoadhesive microspheres of Forskolin (FSK) for hypertension.

Methods: The microspheres were prepared by orifice ionic gelation method using different ratios of Sodium alginate and Kondagogu gum. Fourier-transformed infrared (FTIR) spectroscopy study shows that drug and other excipients are compatible with each other. A 2^2 full factorial design was applied to optimize the formulation considering concentration of Sodium alginate and Kondagogu gum as independent variables; % swelling index and *In vitro* % muco adhesion as dependent variables.

Results: The prepared formulations were characterized for % yield, drug entrapment efficiency, scanning electron microscopy (SEM) and *in vitro* drug release. Microspheres prepared with the ratio of sodium alginate: kondagogu gum (1:1.5) showed swelling index 37±3 % and *in vitro* mucoadhesion 73±3%. SEM images confirmed that microspheres are a hollow spherical structure to a smooth surface morphology. The drug entrapment efficacy of all the formulations was in the range of 58.42±1.2–79.52±1.5 %.

Conclusion: The values of coefficient correlation (r) were calculated and were found to be zero order release from prepared formulation (K2).

Keywords: Forskolin, Kondagogu gum, *In vitro* % muco adhesion, Swelling index.

INTRODUCTION

Drugs supplied through the buccal route induce a quick onset of effect and enhanced bioavailability. The buccal mucosa offers several advantages for controlled drug delivery for extended periods of time. The mucosa is well supplied with both vascular and lymphatic drainage. Besides, first-pass metabolism and pre-systemic elimination in the gastrointestinal tract are avoided. Furthermore, there is a good potential for prolonged delivery through the mucosal membrane within the oral mucosal cavity [1].

Coleus Forskohlii Extract is an ayurvedic herb. It has been identified as the primary chemical of interest in the plant. Forskolin activates an enzyme cells known as adenylatecyclase. This enzyme increases the level of cyclic AMP which is the most important cell regulating compound in the body. An increased level of cyclic AMP improves circulation, decreases histamine releases and allergic compounds, improves the contraction of heat muscle, relaxes arteries which promote normal blood pressure, increases insulin secretion which in turn supports normal sugar levels in the blood, promotes relaxation of bronchial muscles promoting normal breathing and lastly supports improved fat breakdown [2].

The permeability of the buccal mucosa is 4-4000 times larger than that of the skin. 2 The buccal mucosa is thicker and significantly less permeable than the sublingual area. It is usually not able to supply the quick absorption and good bioavailability seen with sublingual administration. The buccal mucosa has an extent of smooth muscle (non-keratinized) and relatively immobile mucosa which makes it a more desirable region for oral transmucosal drug delivery.

Thus the buccal mucosa is further suited for sustained delivery applications, delivery of less permeable molecules, and possibly peptide drugs. Similar to any other mucosal membrane, the buccal mucosa as a site for drug delivery has limitations as well. One of the major disadvantages associated with buccal drug delivery is the low flux which results in low drug bioavailability [3].

Thus in this study an attempt was made to prepare buccal mucoadhesive microspheres of fors kolin. The mucoadhesive microspheres were characterized by *in vitro* methods for controlled release.

MATERIALS AND METHODS

Materials

Forskolin (FSK) with purity of > 98 % was obtained from Madvik Labs, Hyderabad, India. Sodium alginate and Kondagogu gum were purchased from loba chemie (Bombay). All other chemicals and solvents were of reagent grade or higher.

Preparation of microspheres by orifice-ionic gelation method

Microspheres containing FSK were prepared employing sodium alginate in combination with Kondagogu gum as natural polymers. An orifice-ionic gelation process that has been extensively used to prepare large alginate beads was employed to prepare the microspheres [4, 5].

A two-factor, two-level factorial design (2^2) was employed for an optimization procedure. The values of two coded levels of two factors were assumed after preliminary trials. Design-Expert® 9.0.3 Software was used for the generation and evaluation of the statistical experimental design. Sodium alginate and Kondagogu gum in various proportions (table 1) were dissolved in purified water (30 ml) to form a homogeneous polymer solution. The FSK (1.0 g) with 2 ml of ethanol was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (10% w/v) solution (40 ml) through a syringe 22 gauge, 2 ml of glutarodehyde (cross-linking agent) was added to polymer dispersion and stirred continuously at 200 rpm, 35±1°C for 5 h on magnetic stirrer. The formed droplets were retained in the calcium chloride solution to complete the curing reaction and to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with deionized water, finally dried at 40 °C for 3 hours in a hot air oven.

Evaluation of prepared microspheres

Determination of loading efficiency and yields of production

Loading efficiency (%) = (actual drug content in microparticles/ theoretical drug content) × 100

The yields of production of microcapsules of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microcapsules and percent production yields were calculated as per the formula mentioned below [6].

Production Yield = Practical mass (microcapsules) × 100
Theoretical mass (drug with polymers)

Table 1: Composition of different coded values in 2^2 full factorial design

Formulation	A: Sodium Alginate	B: Kondagogu Gum	Swelling Index %	*In vitro* mucoadhesion %
K1	1	-1	29±2	58±2
K2	1	1	37±3	73±3
K3	-1	-1	22±2	52±3
K4	-1	1	24±3	65±3

Low level (-1); high level (+1): Sodium Alginate (g) 0.25 (-1) and 1 (+1); Kondagogu gum (g) 0.5 (-1) and 1.5 (+1); and mean±SD, *n* = 3.

Shape and size of microcapsules

The average diameters of microcapsules were determined using a caliper (Mitutoyo, Japan) in triplicate and the surface morphology was studied by scanning electron microscope (SEM) (JSM-5310LV Scanning Microscope, Tokyo, Japan)[7,8].

Infrared spectroscopy

IR spectra of the pure drugs and microspheres were recorded using Perkin-Elmer model 883 IR spectrophotometer by making a pellet of the samples with KBr. The resultant spectra was then compared with standard reference (IP 1996) and observe for any type of deviation from the standard.

Physical properties of discs

Each disc contained 150 mg of FSK microspheres. The discs were round and flat with an average diameter of 5±0.1 mm compressed with a constant compression force (2 tones). Hardness of the discs was determined for six discs using Erweka hardness tester (Erweka, Germany). Friability of the prepared discs was assessed using friability tester (Erweka, Germany).

Swelling studies

Upon application of bioadhesive material to a tissue a process of swelling may occur. The swelling rate of buccoadhesive discs was evaluated by placing the discs after weighting (W1) in phosphate buffer solution pH 6.8 at 37 °c. Swelling was measured at time intervals of 15, 30, 60, 90 and 120 min. The disc was removed from the beaker and excess surface water was removed carefully using the filter paper. The swollen disc was then weighed again (W2) and the swelling index was calculated.

Swelling index = (W2-W1)/W1 x 100

***In vitro* mucoadhesion strength**

The sheep stomach mucosa was used for *in vitro* mucoadhesion evaluation. The mucosa was removed and cut into pieces 2 cm long and 2 cm wide and was rinsed with 2 ml of pH 6.4 solution. Fifty microcapsules of each were scattered uniformly on the surface of the stomach mucosa. Then, the mucosa with the microcapsules was placed in a chamber maintained at 93% relative humidity and room temperature. After 20 minutes, the tissue was taken out and fixed on a polyethylene support at an angle 45°. The stomach was rinsed with pH 6.4 hydrochloric acid buffer for 5 minutes at a rate of 22 ml/minutes. The microcapsules adhered on to the surface of mucosa was counted, and the percentage of the adhered microcapsules was calculated [9, 10].

***In vitro* release Studies**

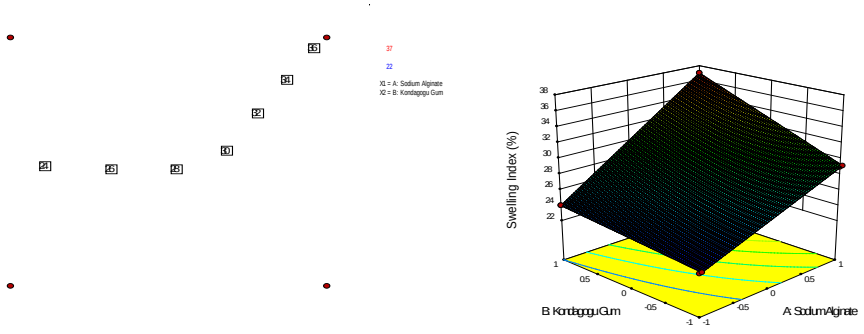
In order to carry out *In-vitro* release studies dissolution test apparatus type II (USP) rotating paddle method was used. The studies were carried out for all formulation combination in triplicate, using 900 ml (37 °C, 100 rpm) of isotonic phosphate buffer (pH 6.8) as the dissolution medium. An aliquot of 5 ml sample was withdrawn at hours intervals and similar volume was replaced with fresh phosphate buffer (pH 6.8) maintained at the same temperature. Samples were then analyzed at 224 nm with UV spectrophotometer.

RESULTS AND DISCUSSION

Microspheres of FSK with various concentration alginate and Kondagogu Gum could be prepared by Orifice-Ionic Gelation technique. The microspheres were found to be discrete, almost spherical, free flowing and of the monolithic matrix type. The purpose of using a full 2^2factorial experimental design was to conduct a comprehensive study of the effect of polymers and their interactions using a suitable statistical tool (Design-Expert® 9.0.3 Software) by applying ANOVA at 0.05 levels and the following models were obtained:

Final equation in terms of actual factors

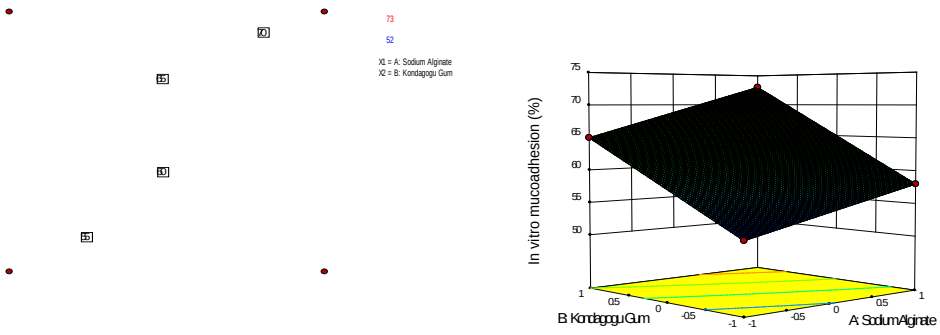
Swelling Index (%)=+28.0+5.0* Sodium Alginate+2.50 * Kondagogu gum+1.50*SA* Sodium Alginate *Kondagogu gum



(a) **(b)**

Fig. 1: Effect of polymers on Swelling Index (%) presented by response surface plot (a), and contour plot (b)

In vitro mucoadhesion (%)=+62.00000+3.50000* Sodium Alginate+7.00000* Kondagogu Gum+0.50000* Sodium Alginate * Kondagogu Gum



(a) **(b)**

Fig. 2: Effect of polymers on *In vitro* mucoadhesion (%) presented by response surface plot (a), and contour plot (b)

From the ANOVA results of the model relating Swelling Index (%) and *In vitro* mucoadhesion (%) as response, it can be noticed that all the coefficients of this model equations had statistical significance (p<0.05) with the R^2 values of 0.9991 and 0.9997. The three-dimensional response surface graphs (fig. 1(b), fig. 2 (b)) and corresponding two-dimensional contour plots (fig. 1(a), fig. 2 (a)) were generated by the Design-Expert® 9.0.3 Software.

The production yields of prepared formulations were in the range of 68.5%-82.26%. This high yield of production is may be due to all the polymer is available for gelation into cross linking agent. Percentage loading efficiency indicated uniformity of drug content in each batch of microspheres. All formulations consisted of 91.29-97.19% drug content, 17.2±0.14-19.28±2 N hardness, and 4.5±0.4-6.15±0.3 % friability (table 2).

Table 2: Evaluation parameters of FSK microspheres

Variable	Formulation code			
	K1	K2	K3	K4
Loading Efficiency (%)	79.52±1.5	58.42±1.2	62.24±1.2	79.34±1.2
Yields (%)	75.8	82.26	68.5	79.22
Mean particle size (µm)	13.92±0.5	11.52±0.4	12.9±0.7	11.52±0.4
Hardness (N)	19.28±2	17.2±0.14	17.8±2	18.9±1.14
Friability (%)	6.15±0.3	5.15±1	4.5±0.43	4.5±0.4

±SD-Standard deviation for (n=3), FTIR spectra analysis revealed no significant interaction between various rational combinations containing physical mixture of drug with polymers as shown in (fig. 3, 4).

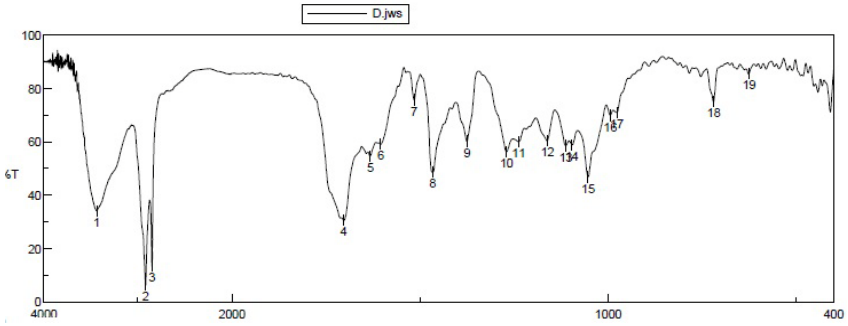


Fig. 3: FTIR spectrum for Forskolin

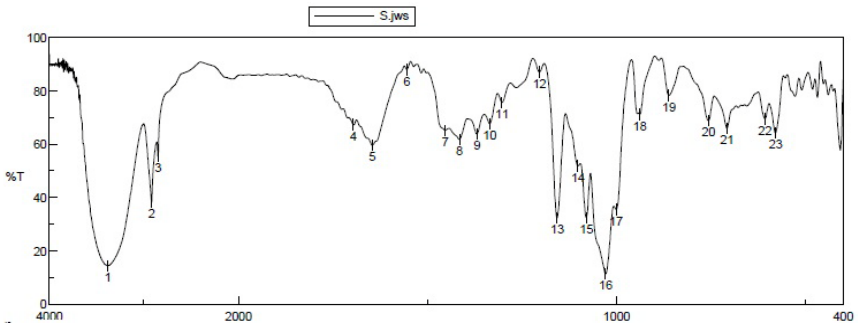


Fig. 4: FTIR spectrum of drug and other ingredients

The microspheres were uniform in size, with a size range of 11.52±0.4-11.52±0.4 µm. Surface morphology of microsphers is presented in fig. 5. The difference in the shape of microcapsules is observed, representing that microsphers containing the higher amount of alginate (K1 and K2) are more spherical and regular as compared to that of microsphers having lower percent of alginate. Such results may be due to as the polymer (alginate) concentration increases the spherical nature of microsphers also increases.

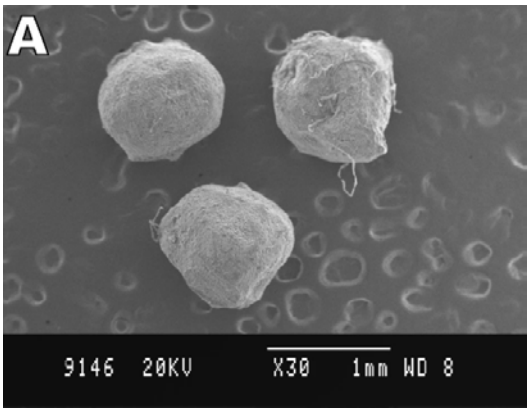


Fig. 5: Scanning electron microscopy of prepared FSK mucoadhesive microspheres

The results of *in vitro* bioadhesive strength study are shown in the table 1. The bioadhesion characteristics were affected by the concentration of the bioadhesive polymers. K2 Formulation showed the highest mucoadhesive property (fig. 6).

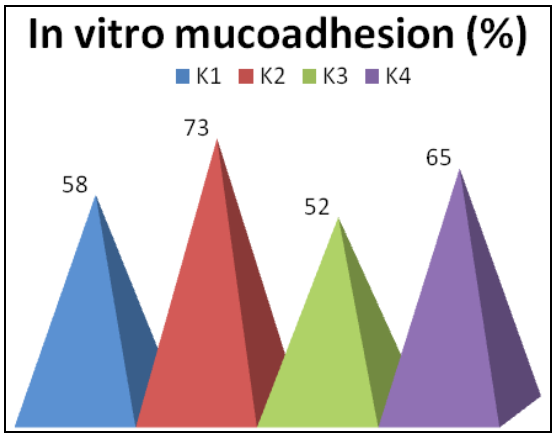


Fig. 6: *In vitro* mucoadhesion (%) of microspheres

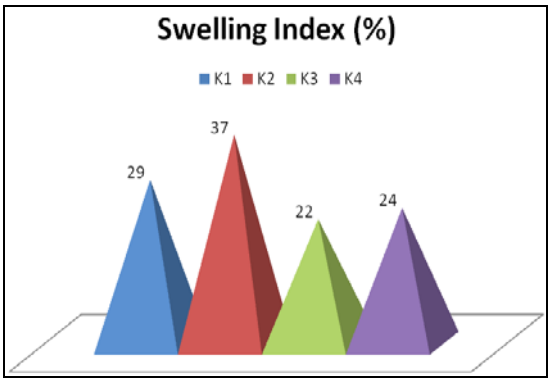


Fig. 7: Swelling index (%) of microspheres

The results revealed that all microsphere formulations swelled rapidly when immersed in 0.2 M phosphate buffer (pH 6.8). After 2 h of incubation swelling percent were observed and represented in table 1. From the results more swelling was observed for more polymer concentration formulations.

In vitro % drug release was conducted and showed in fig 8. Formulation containing Sodium alginate and kondagogu gum (K2) showed controlled release up to 10 h. it was observed that drug release retarded with increasing the polymer concentration. Formulations with higher concentration of kondagogu gum showed rapid swelling and fast release with first order kinetics within 6 h. K2 formulation showed drug release up to 10h with controlled manner due to its high concentration of polymer which gives high swelling and diffuses the drug with the constant rate in to the dissolution medium.

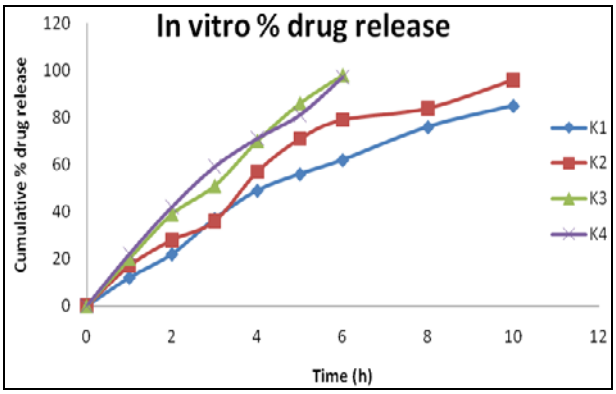


Fig. 8: *In vitro* % drug release from FSK Microspheres formulation

CONCLUSION

The matrix type microspheres prepared successfully by ion-gelation method. Swelling index results, concluded that higher concentration of kondagogu gum gives more swelling. Formulations with high concentration of sodium alginate with kondagogu gum showed more mucoadhesion strength and showed controlled drug release up to 10 h. From the results, it was concluded that FSK microspheres formulation was effective for the treatment of hypertension.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest

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